The Gut Microbiota and Disease: Segmented Filament Bacteria Exacerbates Kidney Disease in a Lupus Mouse Model

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Introduction

Background:
Commensal organisms are a vital component to the host organism. Segmented Filament Bacteria (SFB) is a commensal bacterium found in many animal species. SFB has been shown to exacerbate inflammatory arthritis but has not been implicated in Systemic Lupus Erythematosus (SLE). The focus of our study was to understand the broader relationship between the gut microbiota and lupus.

Methods:
We used the NZM2410 lupus mouse model that is prone to developing lupus manifestations such as immune complex glomerulonephritis in order to study the effect of gut dysbiosis in lupus. NZM2410 mice were inoculated with SFB (n=10) or without SFB-containing (n=10) fecal homogenates. PCR analysis with SFB primers was used to confirm the presence of SFB after inoculation of the mice. Serum was collected monthly, and mice were euthanized at 30 weeks. Kidney damage was accessed using histopathological stains. Immunohistochemistry (IHC) was performed on kidney and small intestine tissues to assess the presence of specific immune cells as well as permeability integrity of the intestine. To analyze changes in the microbiome of SFB colonized mice, fecal bacterial DNA was analyzed using 16S rRNA sequencing.

Results:
Significantly increased glomeruli size was observed in SFB-inoculated mice using H&E staining. PAS staining showed more hyaline deposits in SFB-exposed mice, and Jones staining revealed spike formations in the glomerular basement membrane of SFB mice; all of which are indicative of lupus-like glomerulonephritis. Furthermore, we observed an increased level of fluorescence intensity of C3 and IgG in the glomeruli of SFB-inoculated mice. Inflammatory myeloid cells were also increased in the kidney of SFB mice. The tight junctions Claudin 1 and Claudin 3 were decreased in the small intestine of mice inoculated with SFB, suggesting elevated intestinal permeability better known as gut "leakiness". There were significant differences in the intestinal microbial composition of SFB-exposed mice. These differences were evident down to the Genus taxonomic level and either indicated a disease-driven and/or SFB-influenced commensal change.

Conclusion:
These findings suggest that SFB can act as a "pathobiont" in lupus. SFB may have no role in disease in wildtype mice, but our findings suggest that gut microbiota containing SFB can exacerbate the disease progression of lupus in mice.

Clinical Disease is Increased in +SFB Mice

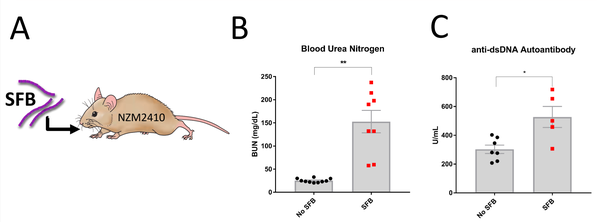

Figure 1: BUN and anti-double stranded (dsDNA) DNA Autoantibody
A. Mice inoculated with SFB fecal homogenates by oral gavage. **B.** Blood Urea Nitrogen levels are increased in mice inoculated with +SFB fecal homogenates. **C.** Anti-dsDNA autoantibody levels are also increased in the same cohort. *p<0.05; **p<0.005.

Increased Glomerulonephritis in +SFB Mice

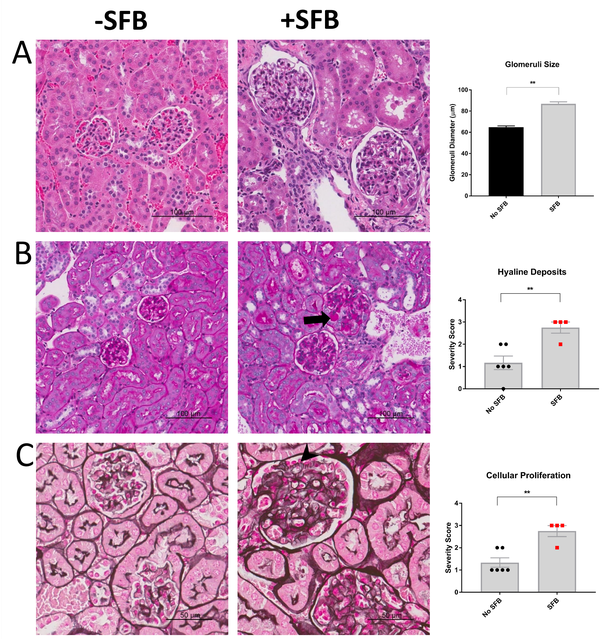

Figure 2: H&E, PAS, and Jones Staining
A. Hematoxylin and Eosin (H&E) staining show increased glomeruli sizes in SFB inoculated mice. **B.** Periodic Acid-Schiff Staining show increased hyaline deposits (arrow) in SFB inoculated mice. **C.** Jones staining show spike formations in +SFB mice (arrowhead). The graph on the right shows increased cell proliferation. *p<0.05; **p<0.005.

↑ Immune Complex Deposition in +SFB Mice

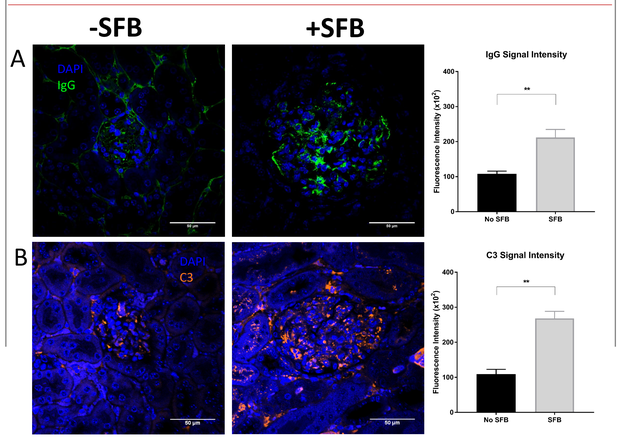

Figure 3: IgG and C3 Deposits in Kidney
A. Glomerular IgG deposits (green) are increased in +SFB mice. Standardized fluorescence intensity analysis is shown at the right. **B.** Glomerular C3 deposits (orange) are increased in +SFB mice as well. Standardized fluorescence intensity analysis is shown at the right. **p<0.005.

↑Inflammatory Cytokine Levels in +SFB Mice

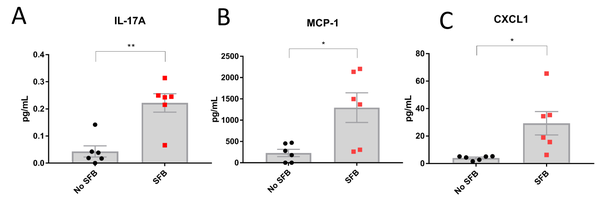

Figure 4: Cytokine Levels in Serum
A. IL-17A, **B.** MCP-1, and **C.** CXCL 1 cytokine concentrations are increased in +SFB mice compared to –SFB mice. *p<0.05; **p<0.005.

Macrophage and Tight Junction Abberations in +SFB Mice

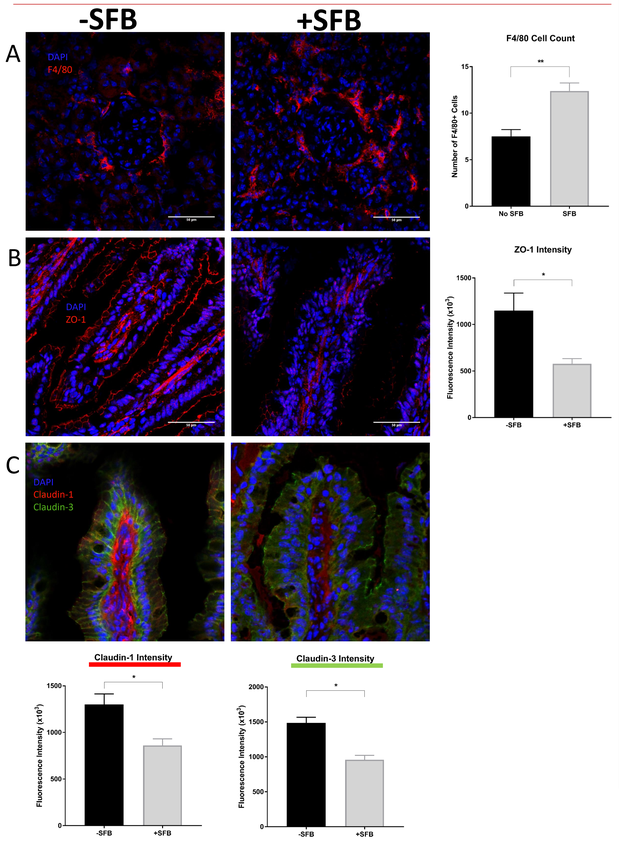

Figure 5: Small intestine IHC staining
A. F4/80+ macrophages (top panels; red) are increased in +SFB mice. **B.** ZO-1 tight junction scaffolding proteins (middle panels; red) were decreased in +SFB mice. Standardized fluorescence intensity analysis is shown at the right. **C.** Claudin 1 (bottom panels; red) tight junctions and Claudin 3 (bottom panels; green) tight junctions were also decreased in +SFB mice. Standardized fluorescence intensity analysis for Claudin 1 and Claudin 3 are shown. *p<0.05; **p<0.005.

Gut Microbiome 16S rRNA Heat Map

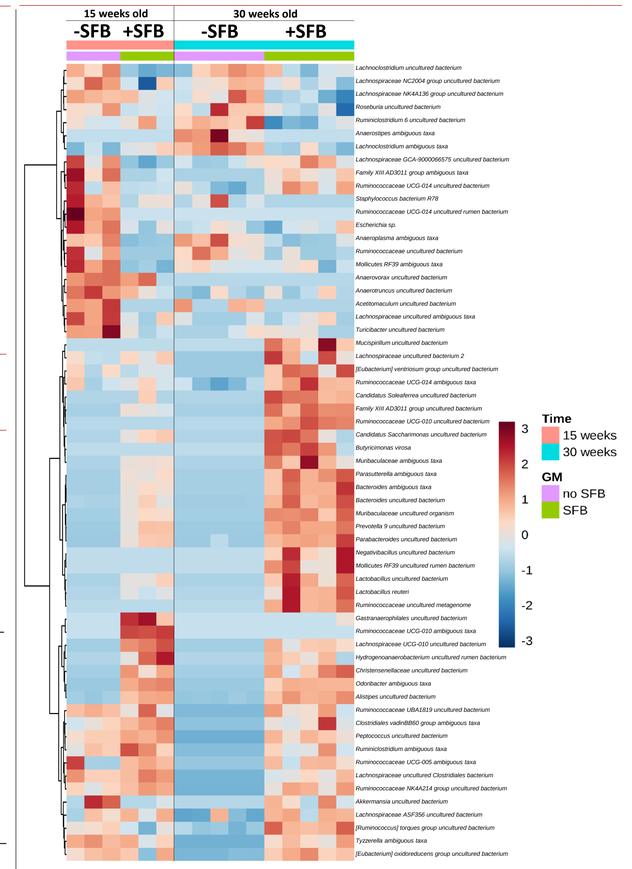

Figure 6: Gut microbiome 16S rRNA sequencing of +/-SFB mice is compared between 15 and 30 week old NZM2410 mice. The genus taxonomic level is represented in this heat map as relative abundance operational taxonomic units (OTUs). All bacterial genera listed are statistically significant (p<0.05 with multivariate analysis).

Conclusion

Key Points
- Mice inoculated with fecal homogenates containing SBF acquire worse kidney disease than -SFB mice.
- Macrophage and tight junction aberrations are present in +SFB mice.
- Gut microbiome patterns are observed with or without SFB and disease progression/time.

Significance
- Disease exacerbation influenced by the gut microbiota supports the idea that environmental factors can affect lupus.

Future Studies
- House lupus mice in a germ-free environment to understand the relevance of the gut microbiota to disease progression.
- Inoculate lupus mice with monocolonized SFB fecal homogenates to observe the role that SFB plays in disease progression.
- Inoculate lupus mice with monocolonized SFB fecal homogenates at different age points to test whether disease progression is exacerbated at a specific time point(s) after inoculation.